Oxygenic phototrophic biofilms for improved cathode performance in microbial fuel cells

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Capturing electrons in the cathodic chambers of microbial fuel cells (MFCs) is a typical limiting aspect of its performance. Recently, research on biocathodes has gained more interest as it allows circumventing the utilisation of exogenous and unstable mediators at a lower cost. It is shown here that the growth of oxygenic phototrophs as a biofilm, increases the current output by two fold. This was possible by forcing the biofilm to grow directly onto the cathode, thus, producing the oxygen directly where it was consumed. This enhancement of the cathodic efficiency was stable for over 30 days.

Key words: Microbial Fuel Cell, biocathode, oxygenic photosynthesis, biofilm.

1 Introduction

Microbial fuel cells (MFC) are energy transducers comprising an anode and a cathode and typically a cation exchange membrane. In such systems, anaerobic electroactive microorganisms use the anode electrode as an electron acceptor when mineralising organic matter. The resultant electrons pass through an external circuit before arriving at the cathode, where they react with a compound of a higher redox potential (e.g. oxygen, ferricyanide) and cations, thus producing current. The first MFC demonstration was achieved in 1911 [1], but research in the subject has really thrived in the last 20 years and especially this last decade.
[2], during which a diversity of MFC embodiments, and a variety of parameters depending on the target applications have been demonstrated [3,4]. Recent research has implied the use of phototrophs either as the electron provider for the anode [5,6], or as a potential electron acceptor in the cathodic compartment. In this work we will focus on the use of phototrophs as the catalyst in biocathodes.

In conventional MFCs, oxygen reacts with electrons flowing from the cathode, and power outputs are, thus, limited by the high overpotential of oxygen. To overcome this, mediators or catalysts are usually required [7], but with the added problems of increased material costs and hindered sustainability through time. For this reason biocathodes have recently attracted great interest since they can increase power output at a lower cost and with better sustainability [8,9]. The essence of biocathodes is to utilise microorganisms as biocatalysts to mediate the reduction of an oxidant either directly or indirectly [10-12]. One of the numerous possibilities is the use of phototrophs [13].

A first study, performed by Cao et al., has shown that anoxygenic phototrophic mixed cultures dominated by *Rhodobacter* and *Rhodopseudomonas* (α-Proteobacteria), previously grown on the anodic part of an MFC, were able to accept cathodic electrons and use them for CO$_2$ carbon fixation in a light dependent manner [14]. As indicated in a recent review, the results of Cao et al. do not specifically demonstrate phototrophic microorganisms to be responsible for the electron uptake [12]. In a more recent study, Powell et al. have coupled the algal cathodic half-cell (*Chlorella vulgaris*) to a yeast anodic half-cell [15]. They have showed that such an MFC was producing a power density of 0.95 mW.m$^{-2}$ at 90 mV, with a load of 5 kΩ. The algae were under agitation and not in direct contact with the cathode, and 2-hydroxy-p-naphthoquinone (HNQ) was used as a mediator. In this study, it was suggested that the electrons from the cathode directly serve the phototrophic organisms to fix CO$_2$. Since the microorganisms employed were oxygenic phototrophs that were releasing O$_2$ from the hydrolysis of their electron’s source (H$_2$O), it is difficult to decipher if it was the oxygen present in the catholyte that reacted with the electrons through the mediator, or if it was the
phototrophs themselves [15,16], especially since air was pumped into the catholyte “providing oxygen and CO₂” [15]. Nevertheless, it has been shown that *in-situ* phototrophic biofilms formed on an electrode immersed in a river stream, were able to catalyse the oxygen reduction thus, showing that biofilms could be more appropriate than a suspension of planktonic cells, as suggested by Huang *et al.* [17] [10].

The aim of the current study was to continue investigations in the field of cathodic efficiency enhancement, with the objective of growing a biofilm of mixed oxygenic phototrophs onto the cathode. The hypothesis is that cathode efficiency can be improved by directly producing the oxygen where it is consumed, thus, avoiding the use of any mediators. This follows from the observation of oxygen-supersaturation in stratified ecosystems, such as in microbial mats, can reach 5-fold higher concentrations [18-20]. The stability of current output was also monitored in order to investigate if such a system could be useful for MFC applications where oxygen is a limiting factor [8,10] (Fig. 1).

2 Material and Methods

2.1 Strain and culture media

The mixed culture of oxygenic phototrophs used in the cathodic chamber consisted of the cyanobacteria *Synechococcus leopoliensis*, *Anabaena cylindrica* and the algae *Chlorella pyrenoidosa* (obtained from “www.sciento.co.uk”). The main reasons for the selection of organisms was to have highly active, fast growing algae and cyanobacteria (*Chlorella pyrenoidosa* and *Synechococcus leopoliensis*), but also filamentous cyanobacteria (*Anabaena cylindrica*) that would have facilitated the anchoring of the two other strains in the cellulose matrix. All species were grown separately in BG-11 media. A mix culture was obtained by adding 20mL of 10⁸ cells per mL of each parent-culture in the same catholyte reservoirs of 1L prior to its connection to the MFCs. The catholytes consisted of the same BG-11 medium for freshwater strains as the one used for the growth of the oxygenic phototrophs [21]. The anodic compartments were inoculated with a pure culture of *Shewanella oneidensis* MR-1
The Shewanella oneidensis cultures were directly introduced into the anodic compartment of the MFC and not the anolyte reservoir. The anolytes consisted of the nutritionally rich LB medium [24].

### 2.2 MFCs design and operation

The microbial fuel cells were made of acrylic material and comprised two 25mL compartment separated by a cation exchange membrane (CEM) with a 30 cm² surface area (VWR) as described elsewhere [25,26]. The electrodes in both the anode and cathode were a 270 cm² sheet of carbon fibre veil (20 g m⁻²) (PRF Composite Materials Poole, Dorset, UK) folded down to a 3D structure with an exposed surface area of 5 cm². The cathode’s side facing the outside was covered by a 5 cm² cellulose matrix (DRY-FRESH 800 NL; Sirane Ltd, Telford, UK) as the substratum for the colonisation by oxygenic phototrophs. However, the cellulosic matrix applied in the cMFC had an additional plastic film (high density polyethylene) within interfacial contact with the cellulose sheets, acting as an inhibitor of algal cell attachment. In addition, the cMFC differed from the pMFC by its anode that had a higher surface area (500cm²). Although the anode surfaces were different, open–circuit voltages ($V_o$) of the pMFC and the cMFC could still be compared: $V_o$ reflects the redox potential difference between the two compartments and, thus, is independent of the electrodes surface area [27].

Since they had a different anode surface area, the comparison between the current production of the pMFC and the cMFC (when a load was applied) could only be performed under normalised conditions according to the electrode total macro-surface areas. The photosynthetic MFC (pMFC) was inoculated with the culture of oxygenic phototrophs 3 weeks after the control MFC (cMFC) was inoculated, in order to insure that no oxygenic biofilm was developing on the cathode of the pMFC (high density polyethylene film).

Both anodic chambers were fed from the same 10L anolyte reservoir, but each MFC had a dripping system in order to isolate the two hydrodynamic circuits thus, avoiding fluidic cross conduction of electrons as well as contamination of the anolyte reservoir(Fig. 2). The anolyte
passed through the anodic chamber at a flow of 0.150 mL min\(^{-1}\) and was then discarded (perfusion model [28]). The anolyte was a flow through system, whereas the catholyte was a closed loop system (Fig. 2). In order to force the oxygenic phototrophs to form a true attached biofilm within the cellulose matrix, a high flow of catholyte (24.5 mL min\(^{-1}\) instead of 0.15 mL min\(^{-1}\) for the anolyte) was applied, whilst the 1L reservoir of catholyte was covered with aluminium foil to prevent any algal/bacterial growth (hydraulic retention time (HRT) of 40 min). The tubing consisted of black ISO-Versinic (3 mm ID; Saint Gobain Performance Plastics, FR), thus, the only zone open to light was the cathodic chamber (cathodic HRT of 1 min). The partial pressure of gas in the catholyte reservoirs was in equilibrium with that of the atmosphere through a sterile air filter of 0.45 µm porosity. The MFCs were placed in a light box 50 cm away from a 30W compact fluorescent light bulb (1535 lumens, 6400K daylight) and a 12h dark/light exposure was applied. The temperature was monitored during this experiment and was found to be 27°C ± 2°C for both day and night phases.

3 Results and discussion

After 30 days, only a small proportion of the cMFC’s cathode surface was colonised whereas the pMFC cellulose sheet was entirely covered by an oxygenic phototrophic biofilm (by visual inspection). This allowed the comparison of the impact of a cathodic biofilm of oxygenic phototrophs on the MFC performance, in which case only the pMFC had developed an oxygenic phototrophic biofilm onto its cathode. Open circuit voltages (\(V_O\)) measured during the first 14 days of the experiment indicated that only the pMFC developed a light-dependent behaviour (Fig. 3a). This light response became noticeable 5 days after the reservoir inoculation by the mixture of oxygenic phototrophs (OP) and stabilised after the 6\(^{th}\) day. On the contrary, the \(V_O\) measured in the cMFC, did not indicate any light response: 540.75 ± 6.61 mV between day 5 and day 15. In fact, the \(V_O\) measured in cMFC, under either light or dark treatment, corresponded to the \(V_O\) measured during the dark phases of pMFC (Fig. 1a). During the same period, the daylight \(V_O\) reached a plateau (pMFC), which was
always $25 \text{ mV} \pm 2.27 \text{ mV}$ higher than the night-time one. As those day light $V_O$ variations were light-dependent, they were probably influenced by the activity of the photosynthetic organisms. Since the only phototrophic microorganisms present were the ones performing oxygenic photosynthesis, it can be thus assumed that during the light phase oxygen was produced. In a MFC, open circuit voltage is a reflection of the redox potential between the two half-cells. The fact that the $V_O$ increased during the light phase, suggests that this was the result of a strong oxidant being produced in the cathodic compartment rather than a reducing agent produced in the anode compartment.

A 7.5 KΩ load was applied to both pMFC and cMFC on the 14th day of the experiment (Fig. 3b). Results confirmed that the same light response was observed under current production (Fig. 3b). The loaded circuit voltage ($V_L$) was characterised by a similar pattern between the day steady-state and the night one. However, the differences between those two steady-states increased 6 days after the application of the load. Then, starting from the 7th day, the system stabilised in $V_L$ between night and day steady-states ($\delta V_{\text{day/night}}$) (Fig. 3b). The standard deviation of $V_L$ average values (Tab. 1) shows that the system was very stable during each steady-state. Moreover, in comparison to $V_O$, the $V_L$ was characterised by a higher $\delta V_{\text{day/night}}$ (75.8 mV ± 7.8 mV). Those day-to-day variations were also stable as diurnal oscillations until the end of the experiments, thus, showing consistency through time.

The methodology and results published by Powell et al. being the closest to the present study and containing the appropriate information, allowed a valid comparison [15]. They reported, for the maximum power density, a voltage of 90 mV with a 5 kΩ resistance that corresponds to 18 µA and 1.62 µW. In the present study, the highest voltage measured was 157 mV with a load of 7.5 kΩ. However, no polarisation data had been produced and such a load should not allow maximum power transfer. Nevertheless, these findings correspond to a current of 20.9 µA, calculated using Ohm’s law ($I = V_L/R$), and a power ($P$) of 3.29 µW ($P = I \times U$) (Tab. 2).

Thus, the values obtained are in the same range as in the Powell et al. study or as reported for
a nitrate-based biocathode [15,29]. In order to give values that could be compared, we calculated the current density normalised either on the Total Macroscopic Surface Area (TMSA, correspond to the unfolded surface area of the anode) or on the Projected Surface Area (PSA, corresponding to the geometric surface area of the folded anode) (Tab. 2). As shown, the values vary considerably and are obviously 53 fold higher when using the PSA. Because the anodic microorganisms used the anode in direct contact to transfer their electrons [23], the TMSA is more representative in terms of power output for a mediator-less MFC [30]. The PSA remains pertinent in regard to the surface footprint occupied by the whole MFC apparatus for practical application purposes.

As the system was stable over the final 9 days of the experiment, the $V_L$ values of each time point were averaged and superimposed in order to represent a 24 h cycle characterising the $p$MFC and $c$MFC, with voltage measurements taken every two minutes (720 pts per day). In this instance, each day is considered as a replicate that reflects day to day variations. Then, using Ohm’s law, the current density of a representative 24 h cycle was calculated (Fig. 3c). The current density of the $c$MFC, during both the light and dark phases, corresponded to the dark phase of the $p$MFC (Fig. 3c), thus, confirming a positive increase of current by the oxygenic biofilm. The positive increase of current production in response to illumination was relatively fast (Fig. 3c): in 67 min 90 % of the day steady-state current output increase was reached, with 50 % the first 18 min $\pm$ 1 min. However, it took 6h30 for the light steady-state to be reached (Fig. 3c).

4 Conclusions

In summary, the results presented in the current study confirmed that i) the presence of an oxygenic biofilm enhanced the current produced by the $p$MFC only upon illumination, and ii) this effect was stable over time. These results suggest that the enhancement of the MFC power output by the presence of an oxygenic biofilm could be due to the oxygen supersaturation effect always observed in stratified ecosystems [18-20] (Fig. 4). Future
development of biocathodes based on oxygenic biofilm would imply deeper investigations like oxygen micro-profiling into the biofilm in order to confirm if this enhancement is due to an oxygen supersaturation effect or not.

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Notes and references

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Tab. 1: Differences between day and night $V_L$ plateaus of the $p$MFC. The hours in parentheses indicate the period covered by the average calculations. The standard deviation (STDV) accounts for all the points (1 every 2 min) of the period covered by the voltage average. The averaged STDV accounts for the 9 days (day 22 to 30).

<table>
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<tr>
<th>Day</th>
<th>Night Voltage average (00:01 to 05:41) (mV)</th>
<th>STDV (mV)</th>
<th>Day Voltage average (14:01 to 18:05) (mV)</th>
<th>STDV (mV)</th>
<th>Day/Night difference (mV)</th>
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<td>61.466</td>
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<td>154.233</td>
<td>1.265</td>
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<td>157.114</td>
<td>7.210</td>
<td>75.784</td>
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Tab. 2: The highest current and power density values, of the pMFC, normalise either by the Total Macroscopic Surface Area (TMSA) or by the Projected Surface Area (PSA). The day voltage plateau was 157 mV with a load of 7.5 KΩ. The electrodes surfaces were of 270 cm² (TMSA: total macroscopic surface area) or 5 cm² (PSA: projected surface area).

<table>
<thead>
<tr>
<th></th>
<th>Absolute</th>
<th>TMSA (m²)</th>
<th>PSA (m²)</th>
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<td><strong>Current</strong> (mA)</td>
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<td><strong>Power</strong> (mW)</td>
<td>0.0033</td>
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Figure captions

**Fig. 1:** Diagram of a microbial fuel cell. During organic matter mineralisation, the electron is transfer by the biofilm (*Shewanella oneidensis*) to the anode. Electrons flow through an external circuit and protons flow through the membrane. The protons then react with the electrons at the cathode and reduce oxygen into water. In this work, the oxygen is provided by the oxygenic phototrophic biofilm that grow directly on the cathode.

**Fig. 2:** Microbial fuel cell experimental setup. This diagram illustrates the recycled flow of catholyte and the passing through of the anolyte. The anodic compartment is separated from the reservoir of anolyte, by a dripping system, to prevent any contamination. Each MFC had its own catholyte reservoir. As both MFCs have the same anolyte reservoir, they have their own dripping system.

**Fig. 3:** Voltage and power curve of the setup. a) Open circuit voltage of the MFCs. The cMFC (inoculated with oxygenic microorganisms but without any biofilm. The minor variations observed for the cMFC are due to the diurnal temperature cycle. The pMFC (containing a cathodic oxygenic biofilm comprising of *Synechococcus leopoliensis*, *Anabaena cylindrica* and *Chlorella pyrenoidosa*) voltage clearly demonstrates a light dependant response. b) Loaded circuit voltage (7.5kΩ) showing the positive light response of the pMFC and the stability over time of its current production. c) Current density of the pMFC and the cMFC over an average 24h diurnal cycle, calculated by the superimposed current measured from day 21 to day 30 (load of 7.5kΩ), where the cathodic oxygenic phototrophic biofilm current increase during light phase is clearly shown.

**Fig. 4:** Local oxygen supersaturation. This diagram illustrates the suggested principle
behind the light-dependent current increase: when in steady-state conditions, because of an oxygen diffusion limitation caused by the oxygenic biofilm, as in microbial mats, a zone of high oxygen concentration appears within the biofilm. As the biofilm covers the cathode, a higher oxygen concentration is available for accepting incoming electrons.
Figure 1
Figure 2
Figure 3
Figure 4